REMARKS

Amendments to the Specification

Applicant has amended the specification to acknowledge government funding. Applicant has also amended the specification to remove inadvertent highlighting that occurred in the original at page 54, line 10 (paragraph 189).

Amendments to the Claims

Claims 1-30 are currently pending. By way of this amendment, applicant has canceled claims 3 and 17 and has amended claims 1, 2, 10 and 12. Upon entry of this amendment, claims 1-2, 4-16 and 18-30 will be pending.

Claims 1 and 2 have been amended to more particularly recite the host cell in which the methods of the invention may be practiced. Support for these claim amendments can be found, e.g., in paragraphs 28, 155-161 and 187 of the application as originally filed. In addition, the limitations of claim 3 have been imported into amended claims 1 and 2 to more particularly recite the N-glycans that are produced according to the claimed methods, and claim 3 has been canceled. Claim 17 has also been canceled for being duplicative of claim 15. Finally, claims 2, 10 and 12 have been amended to correct informalities noted by the Examiner. Each of the claim amendments is supported by the application as originally filed and none adds new matter.

Response to § 112, Enablement Rejections

Claims 1-30 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. According to the Examiner, the application enables yeast host cells that lack och1 gene products and other mannosyltransferases (Office Action at pages 2-3):

> [T]he specification, while being enabling for any yeast lacking och1 and other genes encoding mannosyltransferases, does not reasonably provide enablement for any lower eukaryotic host cell, and specifically not for a yeast cell with no inactivation of a mannosyltransferase.

Applicant traverses in part and for at least the following reasons.

As noted by the Examiner, most yeast and other lower eukaryotic host cells that normally hypermannosylate N-glycans will be useful in the claimed methods only if they lack mannosyltransferase activities responsible for unwanted hypermannosylation. Thus, many embodiments of the invention are directed to engineering human-like glycosylation pathways in lower eukaryotic host cells in which one early step requires inactivating mannosyltransferases. The invention, however, is not limited to such embodiments. Rather, the specification teaches that a broad range of host cells, both lower and higher eukaryotic cells, may be used in the methods of the invention (paragraph 539, pages 79, line 27 – page 80, line 1):

> A preferred host cell of the invention is a lower eukaryotic cell, e.g., yeast, a unicellular and multicellular or filamentous fungus. However, a wide variety of host cells are envisioned as being useful in the methods of the invention. Plant cells or insect cells, for instance, may be engineered to express a human-like glycoprotein according to the invention. Likewise, a variety of non-human, mammalian host cells may be altered to express more human-like or otherwise altered glycoproteins using the methods of the invention.

The specification further teaches that host cells useful in the methods of the invention may be *selected from nature* (i.e., many higher eukaryotic cells) *or engineered* (i.e., most lower eukaryotic cells including yeast and filamentous fungi) to be capable of producing human-like N-glycan core structural intermediates, such as, e.g., $Man_5GlcNAc$:

> The invention provides methods for producing a glycoprotein having human-like glycosylation in a ***non-human eukaryotic host cell***.... [A] eukaryotic host cell ***that does not naturally express, or which is engineered not to express, one or more enzymes involved in production of high mannose structures is selected as a starting host cell.*** Such a selected host cell is engineered to express one or more enzymes or other factors required to produce human-like glycoproteins. (paragraph 155, page 42, lines 24-31; emphasis added)

* * *

> In a preferred embodiment, the ***methods are directed to making host cells in which oligosaccharide precursors are enriched in $Man_5GlcNAc_2$***. Preferably, a eukaryotic host cell is used that does not express one or more enzymes involved in

> the production of high mannose structures. ***Such a host cell may be found in nature or may be engineered, e.g., starting with or derived from one of many such mutants already described in yeasts.*** Thus, depending on the selected host cell, one or a number of genes that encode enzymes known to be characteristic of non-human glycosylation reactions will have to be deleted. (paragraph 162, page 45, lines 5-12; emphasis added)

The application teaches the skilled worker how to engineer or select a lower or higher eukaryotic host cell in which to express a class II mannosidase according to the claimed methods:

> In particular, the methods described herein enable one to obtain, *in vivo*, $Man_5GlcNAc_2$ structures in high yield, at least transiently, for the purpose of further modifying it to yield complex N-glycans. A successful scheme to obtain suitable $Man_5GlcNAc_2$ structures in appropriate yields ***in a host cell, such as a lower eukaryotic organism***, generally involves two parallel approaches: (1) ***reducing high mannose structures*** made by endogenous mannosyltransferase activities, ***if any***, and (2) removing 1,2- α- mannose by mannosidases ***to yield high levels of suitable $Man_5GlcNAc_2$ structures*** which may be further reacted inside the host cell to form complex, human-like glycoforms (paragraph 156, page 44, lines 6-14; emphasis added).
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> Accordingly, ***a first step*** involves the ***selection or creation of a eukaryotic host cell, e.g., a lower eukaryote***, capable of producing a specific precursor structure of $Man_5GlcNAc_2$ that is able to accept *in vivo* GlcNAc by the action of a GlcNAc transferase I ("GnTI"). In one embodiment, ***the method involves making or using a non-human eukaryotic host cell depleted in a 1,6 mannosyltransferase activity*** with respect to the N-glycan on a glycoprotein. Preferably, the host cell is depleted in an initiating 1,6 mannosyltransferase activity (see below). Such a host cell will lack one or more enzymes involved in the production of high mannose structures which are undesirable for producing human-like glycoproteins (paragraph 157, page 44, lines 15-23; emphasis added).

The application then teaches the skilled worker (see, e.g., pages 42-51) various ways to engineer a lower eukaryotic host cell of choice, e.g., by depleting unwanted hypermannosylation reactions, to create a desired host cell which is "capable of producing a specific precursor structure of $Man_5GlcNAc_2$ that is able to accept *in vivo* GlcNAc by the action of a GlcNAc transferase I". $Man_5GlcNAc_2$ is converted to $GlcNAcMan_5GlcNAc_2$ by the action of GlcNAc transferase I (see above quote). Both $Man_5GlcNAc_2$ and $GlcNAcMan_5GlcNAc_2$ are substrates for the class II

mannosidase required in each of the amended claims. Thus the host cells which produce one or both of these substrates are the desired host cells of the pending claims.

Specific examples of ways to produce $Man_5GlcNAc_2$ and $GlcNAcMan_5GlcNAc_2$ N-glycan intermediates in embodiments that use lower eukaryotic host cells are depletion of various known mannosyltransferases and/or their regulators (e.g., pages 45-46 and Tables 1 and 2) from yeast cells, and finding and depleting homologs of such enzymes in, e.g., other yeast and fungal cells. See also page 51 (paragraph 177, lines 7-14), where the specification teaches that host cells may include non-human eukaryotic host cells having reduced *alg* gene activity, and functional equivalents:

> The invention further provides methods of making or using ***a non-human eukaryotic host cell diminished or depleted in an alg gene activity*** (i.e., *alg* activities, including equivalent enzymatic activities in non-fungal host cells) and introducing into the host cell at least one glycosidase activity. In a preferred embodiment, the glycosidase activity is introduced by causing expression of one or more mannosidase activities within the host cell, for example, by activation of a mannosidase activity, or by expression from a nucleic acid molecule of a mannosidase activity, in the host cell.

Thus, the methods of the invention are not, as the Examiner suggests, limited to *och1* and hypermannosylation mutants in yeast. As discussed above, one can also chose a higher (non-human) eukaryotic host cell which is by nature partially or fully "capable of producing a specific precursor structure of $Man_5GlcNAc_2$ that is able to accept *in vivo* GlcNAc by the action of a GlcNAc transferase I" (hence, capable of producing $Man_5GlcNAc_2$ or $GlcNAcMan_5GlcNAc_2$ structures as recited in amended claims 1 and 2) and use such a host cell to express a class II mannosidase according to the methods recited in the pending claims.

Based on the above, applicant has amended claims 1 and 2 (and their dependent claims) to recite that the host cell is "a non-human eukaryotic host cell which produces N-glycans comprising $Man_5GlcNAc_2$ or $GlcNAcMan_5GlcNAc_2$ structures." The amended claims cover embodiments in which a non-human eukaryotic host cell is by nature able to make the N-glycan intermediates upon which the class II mannosidase works; and embodiments in which a lower eukaryotic host cell has been engineered to produce such N-glycans intermediates according to the teachings of the application.

Based on the above, applicant respectfully requests that the Examiner withdraw the enablement based rejection.

Response to § 112, Written Description Rejections

The Examiner has objected to the claim term "lower eukaryotic host cell" as its definition in the detailed description of the invention is broader than the art-recognized meaning, which, according to the Examiner, is a "uni- and multi-cellular fungal and algal cells" (Office Action at page 5), noting that applicant has elsewhere in the application that "some animal or plant cells" do not fall within the definition of "lower eukaryotic host cell". To avoid confusion, applicant has deleted the term from the claims as amended herein, thus obviating this objection.

Response to § 102 Rejections

Claims 1, 5, 7, 15, 17-19, 23, 26 and 27 stand rejected for lack of novelty under 35 U.S.C. § 102(b) over Chiba et al. (*J. Biol. Chem.* 273(41):26,298-304 (1998) ("Chiba")). According to the Examiner, Chiba discloses engineering an *S. cerevisiae* yeast host cell by a method that encompasses expressing a class II mannosidase. Applicant traverses.

Chiba reports the expression in *S. cerevisiae* host cells of a fusion protein comprising an HDEL tag fused to an *Aspergillus saitoi* alpha 1,2- mannosidase, which is a class I mannosidase, not a class II or class III mannosidase. This may be seen in Chiba, Figure 1, middle panel, where alpha 1,2- mannosidase (labeled as "High Mannose Type) is shown to act in the ER. As indicated in that Figure, Class I mannosidases hydrolyze alpha 1,2 linkages and convert $Man_8GlcNAc_2$ structures to $Man_5GlcNAc_2$ structures that are substrates for Golgi localized GnT-I (see also paragraphs 22-28 of the instant application for a discussion of class I mannosidase enzymes and the work of Chiba/Kirin, referenced in Table 2 at page 10, and others).

In contrast, class II mannosidases, such as the alpha-mannosidase II shown to act *after* GnT-I in Chiba Figure 1, and class III mannosidases, hydrolyze alpha-1,3 and alpha 1,6 glycosidic linkages and convert $GlcNAcMan_5GlcNAc_2$ structures to $GlcNAcMan_3GlcNAc_2$ (see, e.g., Figure 1B and paragraph 29 of the instant application). Class II mannosidases are discussed in detail e.g.,

at paragraphs 29-47, 179-531 of the application as originally filed and class III mannosidases at, e.g., paragraphs 48 and 532-538 of the application as originally filed. In addition, Figure 36A shows conversion of $GlcNAcMan_5GlcNAc_2$ to $GlcNAcMan_3GlcNAc_2$ structures by a class II mannosidase, Figure 36B shows conversion of $Man_6GlcNAc_2$ structures to $Man_4GlcNAc_2$ by a class IIx mannosidase, and Figure 36C shows conversion of $Man_5GlcNAc_2$ structures to $Man_3GlcNAc_2$ by a class III mannosidase.

The claims as amended herein specifically require expression of a mannosidase enzyme that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a Manα1,3 and Manα1,6 glycosidic linkage (i.e., a class II or class III mannosidase enzyme). The yeast cells of Chiba do not express either a class II or a class III mannosidase enzyme and, thus, cannot make $GlcNAcMan_3GlcNAc_2$, $Man_3GlcNAc_2$, or $Man_4GlcNAc_2$ structures. Nowhere does Chiba disclose or suggest the method of the instant claims. Accordingly, claims 1-30 are novel over Chiba and withdrawal of the 102(b) rejections in view of Chiba is respectfully requested.

No fee is believed to be due for filing these papers. However, the Director is authorized to charge any fees that may be due to Deposit Account No. 06-1075 (Order No. 099999-0099). A duplicate copy of this paper is enclosed.

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Respectfully submitted,

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